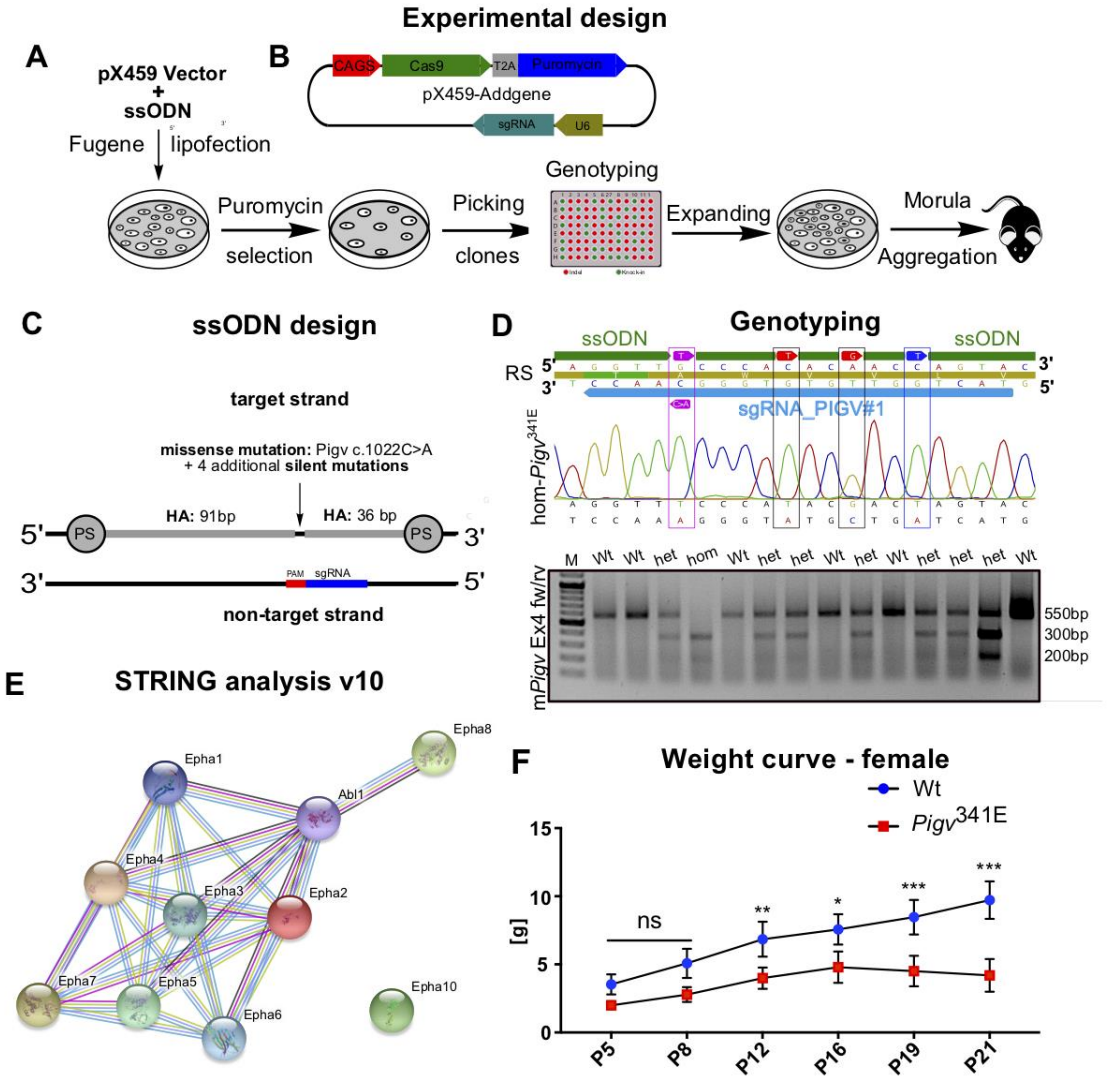
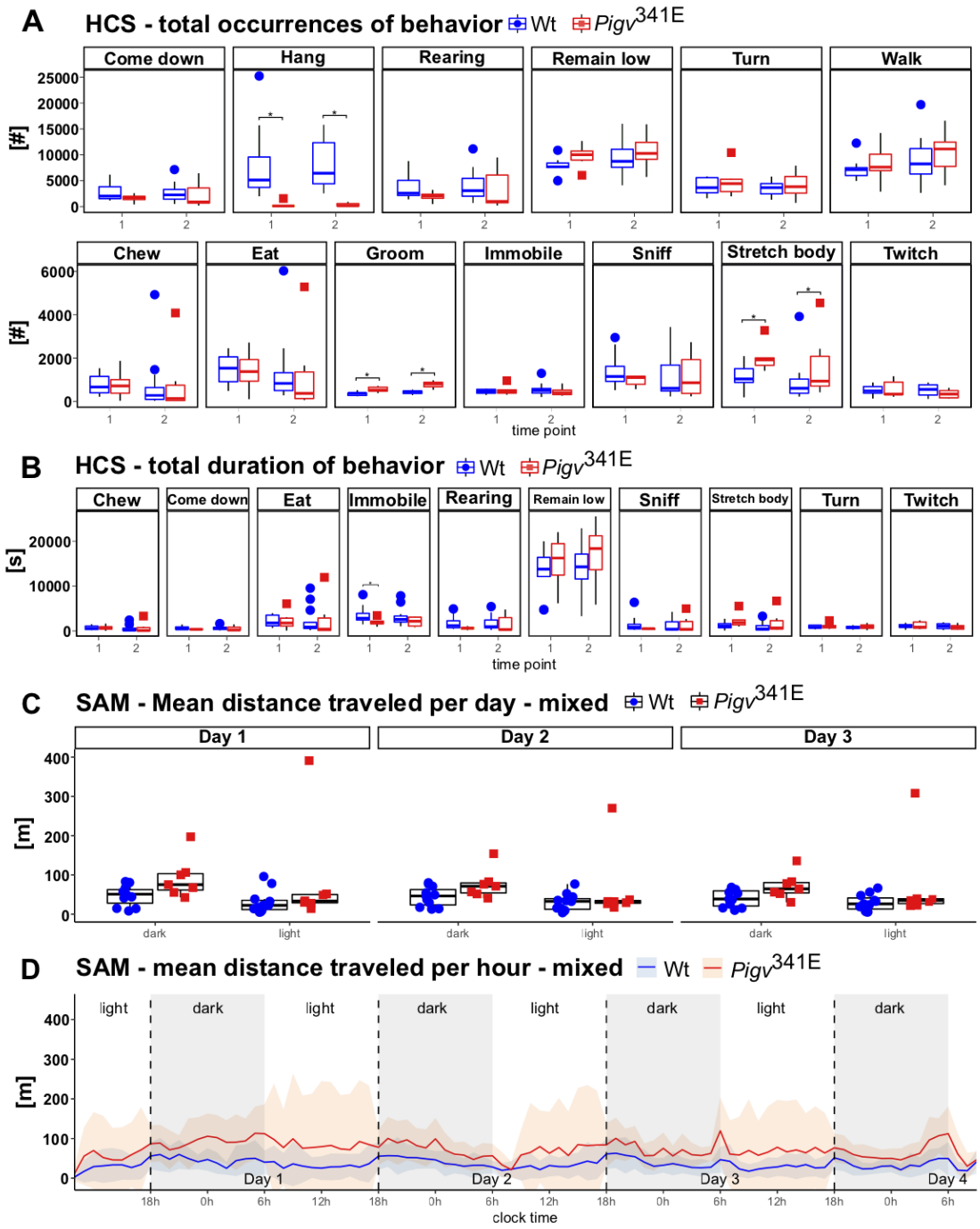
Supplementary figure 1



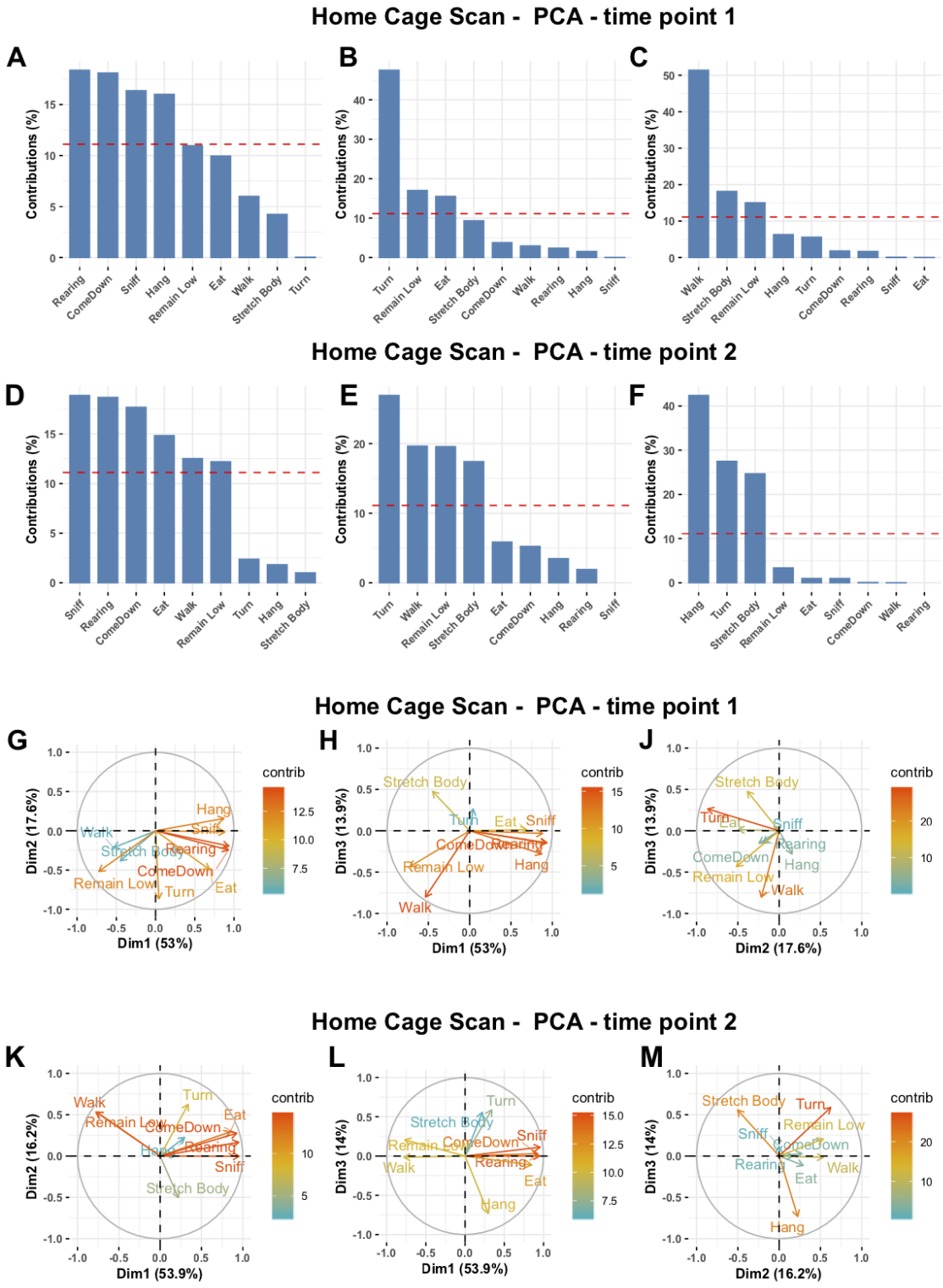
Generation of the mouse model and CRISPR-Cas9 experiments (A) schematic overview of the experimental design from generating the mouse embryonic stem (mES) cell clone to establishment of the mouse model. (B) schematic overview of the pX459 vector from Addgene. (C) single-stranded oligode-oxynucleotides (ssODNs) were designed with asymmetrical homology arms (HA), highlighted in grey and with phosphorothioate (PS) bonds according to Renaud et al. (2016), Richardson et al. (2016). ssODNs were identical to the target strand. Single-guide RNA (sgRNA) is highlighted in blue. Protospacer adjacent motif (PAM) is marked in red (D) ssODNs contained the nucleotide T (marked in pink) causing the missense mutation c.1022 C>A on the complementary strand and three additional silent mutations (marked in red and blue) that avoid a second cut by Cas9 after homology-directed repair (HDR) has happened. The silent mutation marked in blue generated a restriction site for *BcuI* that was used for genotyping purposes. Sanger sequencing of homozygous *Pigv*^{341E} knock-in clone revealed the stable integration of missense mutation (on the (-) strand C>A, on the (+) strand G>T, causing PIGV p.Ala341Glu) and silent mutations (on the (+) strand C>T, A>G, C>T) at CRISPR-Cas9-induced DSB by HDR. Gel electrophoresis identified genotypes in mice by *BcuI* digest of PCR-amplified products using the primers m*Pigv*_Ex4_fw and m*Pigv*_Ex4_rv. RS: Reference sequence of *Pigv* (E) STRING analysis v10 confirmed protein-protein interaction between EphA-receptors and Abl1. Turquoise string: from curated databases, pink string: experimentally determined, yellow string: textmining, black string: co-expression, lila string: protein homology. (F) Female *Pigv*^{341E} mice reveal a reduced weight from postnatal day (P) 1-21. Animals used for the weight curve: Wt(female n=4) *Pigv*^{341E}(female n= 3). The data from the weight curve was analyzed by two-way of variance (ANOVA) followed by a Bonferroni's multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001.

Supplementary figure 2



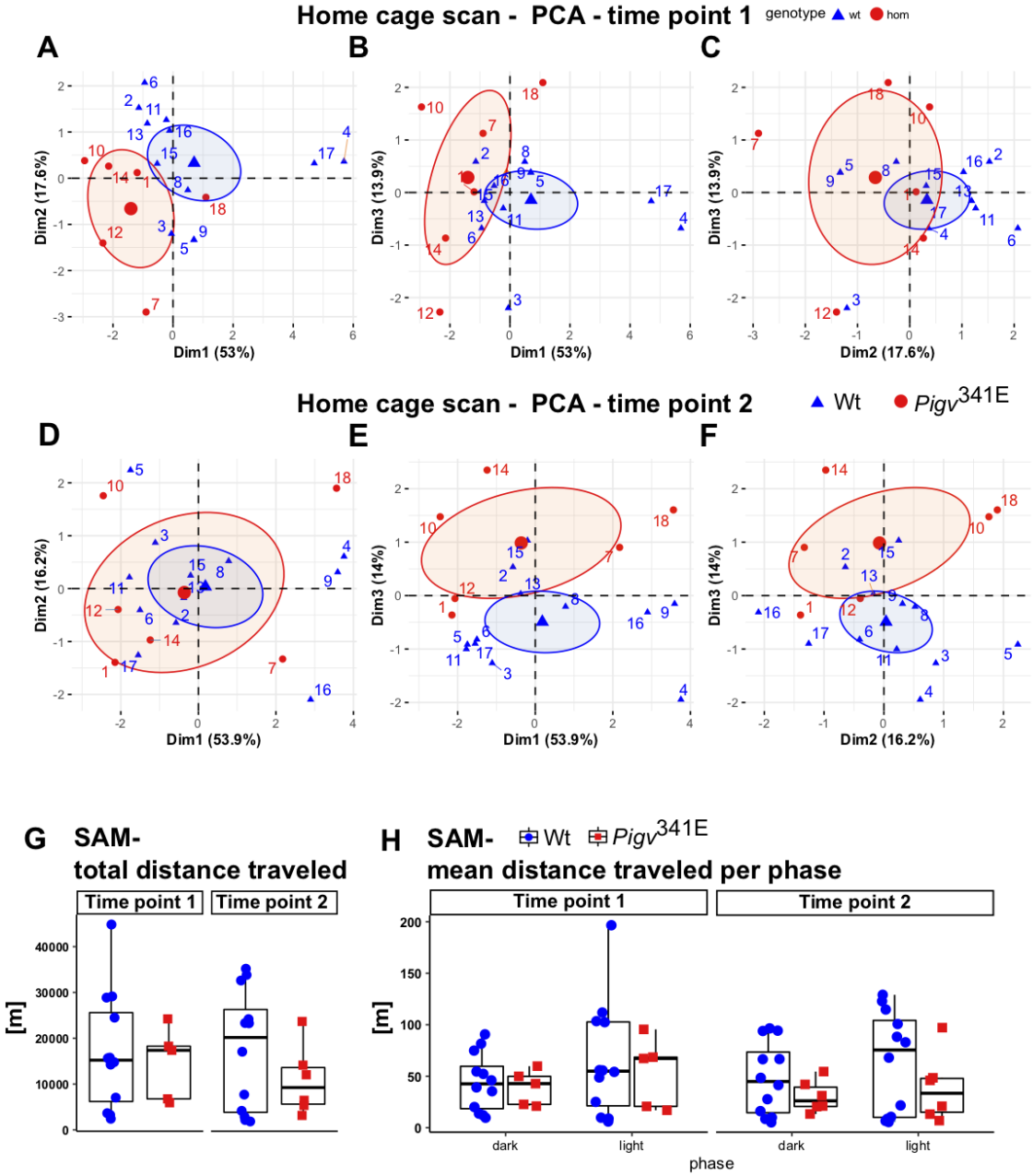
HCS and SAM (second approach) (A) Total occurrences of behaviors from the home cage scan (HCS). *Pigv*^{341E} mice revealed a decreased number of hanging and an increased number of grooming at both time points. *Pigv*^{341E} mice showed an increased number of "stretch body" at time point 1. (B) Total duration of behavior of behaviors from the HCS. *Pigv*^{341E} mice showed a decreased "immobile" behavior at time point 1. (C, D) In the social activity monitor (SAM), no significant differences in mean distance traveled per day and per hour were observed between genotypes when held in mixed genotypes. *Pigv*^{341E}=homozygous for Pigv p.Ala341Glu, wt=wild-type. Animals used for the HCS were at time point 1 (tp1) 8 weeks old, at time point 2 (tp2) 16 weeks old: Wt(female n=8, male n=4) *Pigv*^{341E}(female n= 4, male n=2). Animals used for SAM: Wt(female n=4, male n=6) *Pigv*^{341E}(female n= 2, male n=5). The data from the HCS (total occurrences, total duration) was analyzed with Wilcoxon rank sum test (non parametric). The data from the SAM (distance traveled per hour) was analyzed with generalized linear mixed-effects models (glmm) using a Markov chain Monte Carlo (MCMC).*P<0.05.

Supplementary figure 3



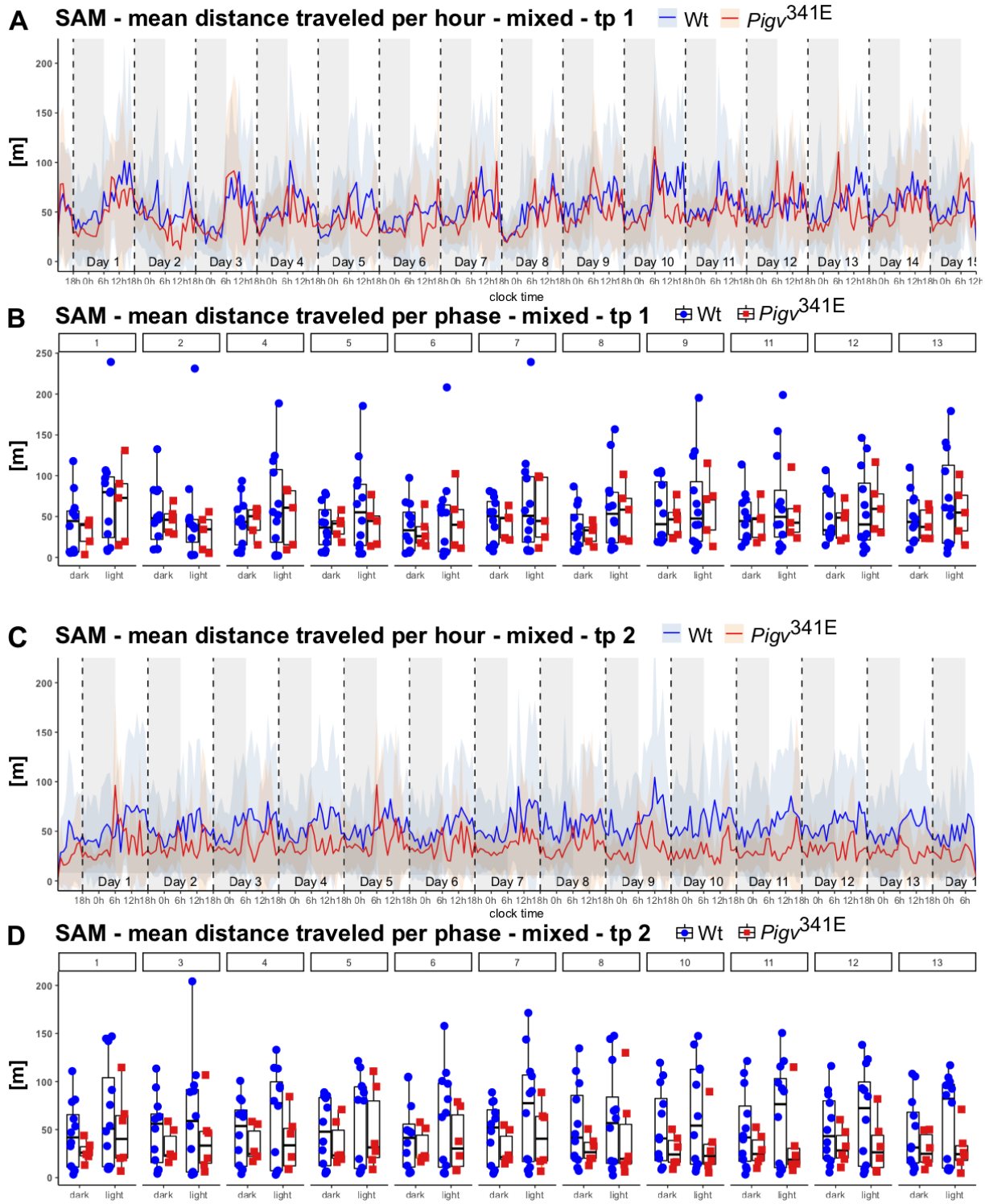
HCS (Home cage scan) – principal component analysis (PCA). (A-F) contribution of original variables (occurrences of behavior) to the first three components of the PCA for each time point. (G-M) 2D Representation of the coefficient of all variables along PC1 and PC2 (left), PC1 and PC3 (middle) and PC2 and PC3 (right).

Supplementary figure 4



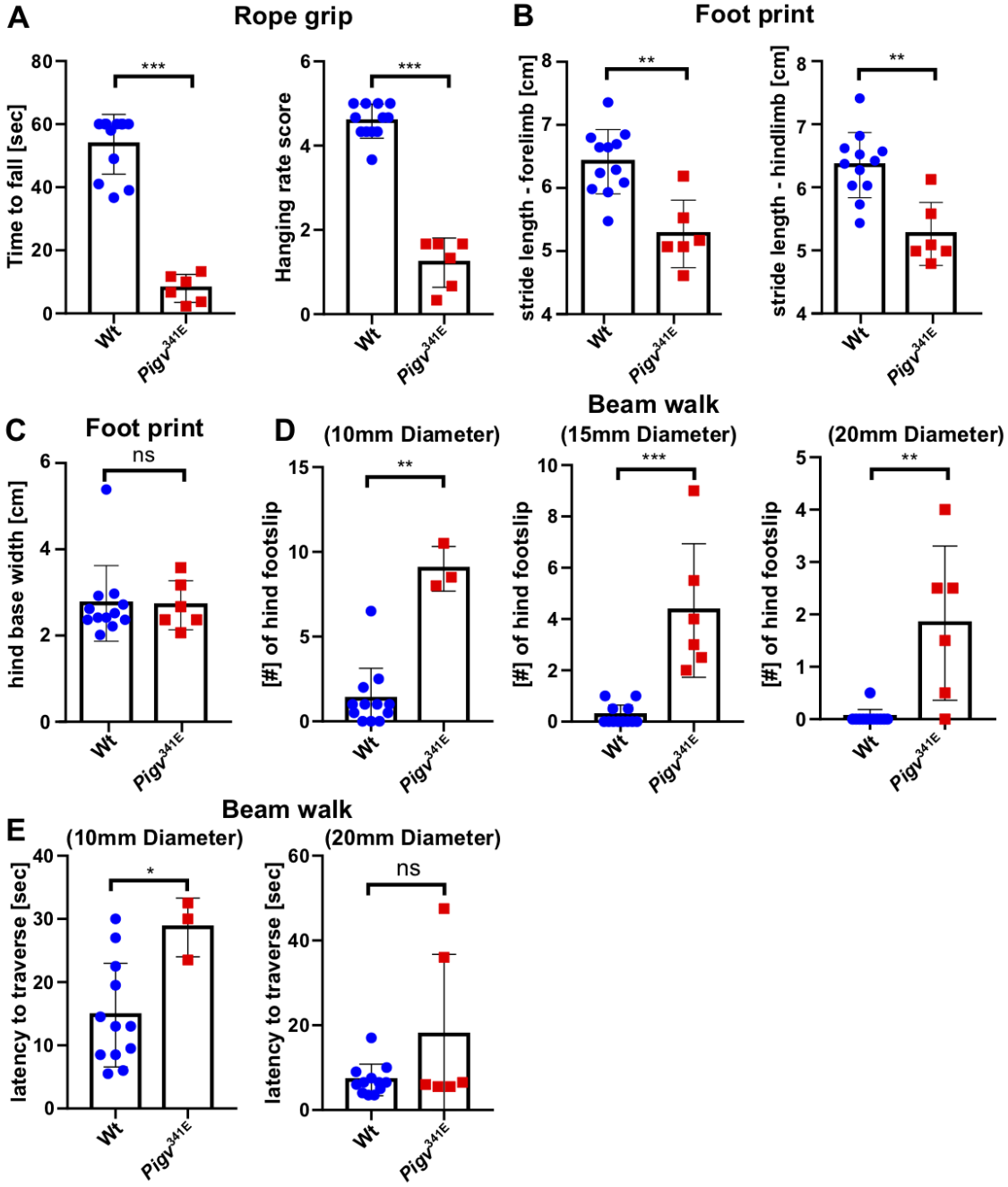
HCS (Home cage scan) – principal component analysis (PCA), SAM (first approach). (A-F) Group separation and individual contribution to PC1 and PC2 (left), PC1 and PC3 (middle) and PC2 and PC3 (right). Mean (bigger symbol) and confidence interval (ellipse) of each group were shown. Genotypes differ significantly in dimension 1 at time point 1 and in dimension 3 in time point 2 (G-H) Social activity monitor (SAM) showed no significant differences in total distance traveled and distance traveled per phase between genotypes when held in mixed genotypes.
Pigv^{341E}=homozygous for Pigv p.Ala341Glu, wt=wild-type. Animals used for the SAM were at time point 1 (tp1) 8 weeks old: Wt(female n=8, male n=4), *Pigv*^{341E}(female n= 3, male n=2). Animals were at time point 2 (tp2) 16 weeks old: Wt(female n=8, male n=4), *Pigv*^{341E}(female n= 4, male n=2). Data from PCA was analyzed with a Wilcoxon rank sum test. The data (distance traveled per hour) from the SAM was analyzed with glmmMCMC.*P<0.05, **P<0.01, ***P0.001.

Supplementary figure 5



SAM (first approach). (A, C) In the social activity monitor (SAM), no significant differences were observed in distance traveled per hour at both time points between genotypes when animals were held in mixed genotypes. Both genotypes were significantly more active during the light cycle than during the dark cycle at both time points. (B, D) No significant differences were observed in distance traveled per phase at both time points between genotypes when animals were held in mixed genotypes. *Pigv*^{341E}=homozygous for Pigv p.Ala341Glu, wt=wild-type. Animals used for the SAM were at time point 1 (tp1) 8 weeks old: Wt(female n=8, male n=4), *Pigv*^{341E}(female n= 3, male n=2). At time point 2 (tp2) animals were 16 weeks old: Wt(female n=8, male n=4), *Pigv*^{341E}(female n= 4, male n=2).

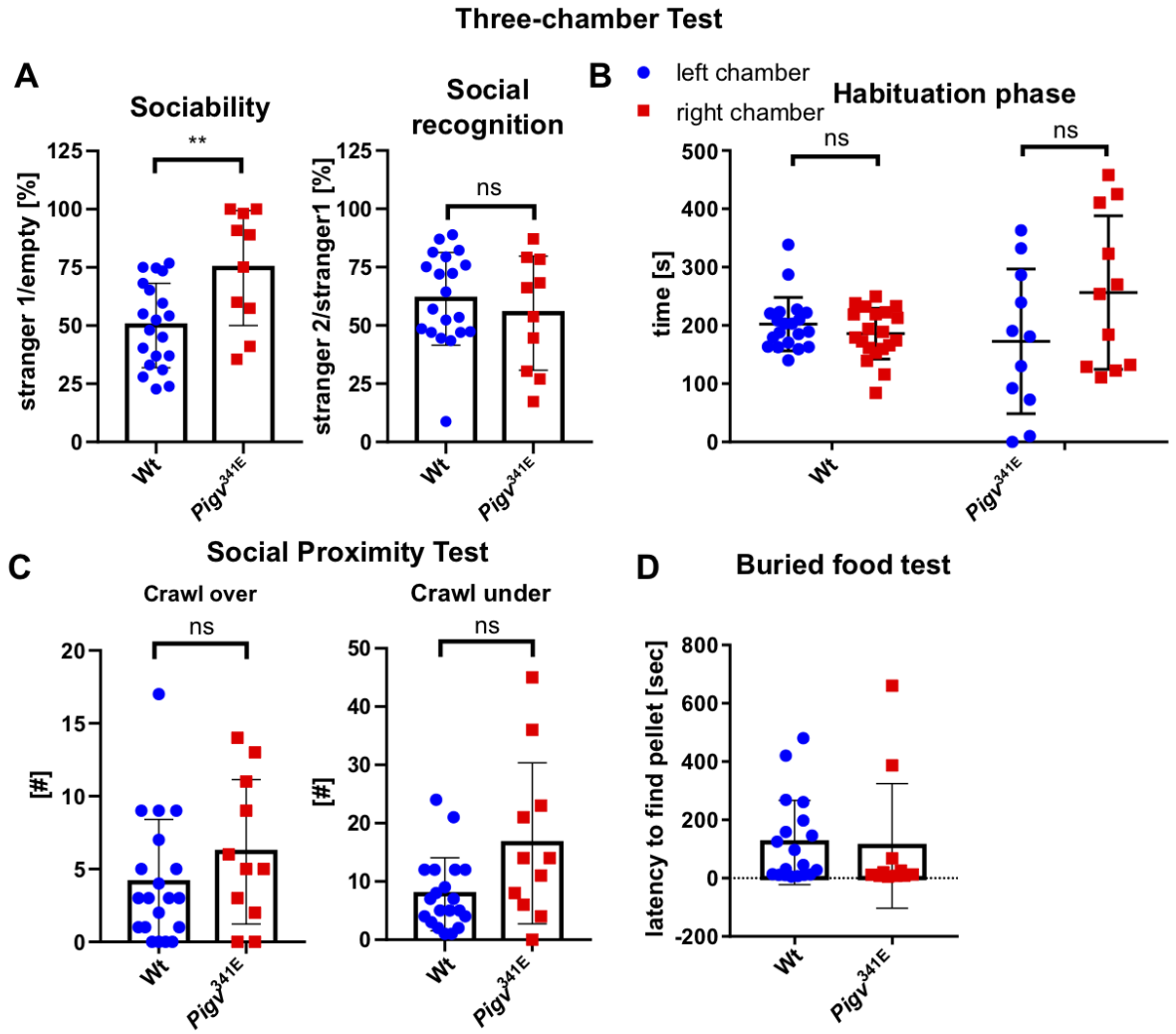
Supplementary figure 6



***Pigv*^{341E} mice show a motoric phenotype** (A) In the rope grip test, *Pigv*^{341E} mice showed a decreased climbing performance displayed by a decreased time to fall off the rope (left graph) and a decreased hanging score (right graph). (B,C) In the foot print test, *Pigv*^{341E} mice exhibited no significant differences to wild-type in hind base width but showed a decreased forelimb and hindlimb stride length (FL-SL, HL-SL). (D) *Pigv*^{341E} mice had deficits in motor coordination displayed by an increased number of hind footslip when walking on beams with different diameters (10-20 mm diameter). (E) *Pigv*^{341E} mice showed an increased latency to traverse a beam with a diameter of 10 mm (left graph) but revealed no significant difference to wild-type mice traversing a beam of 20 mm (right graph).

Pigv^{341E}=homozygous for Pigv p.Ala341Glu, wt=wild-type. Wt(female n=8, male n=4) *Pigv*^{341E} (female n= 4, male n=2). Animals were 7 weeks old. The data was analyzed with a non-parametric t-test (Mann-Whitney). *P<0.05, **P<0.01, ***P<0.001

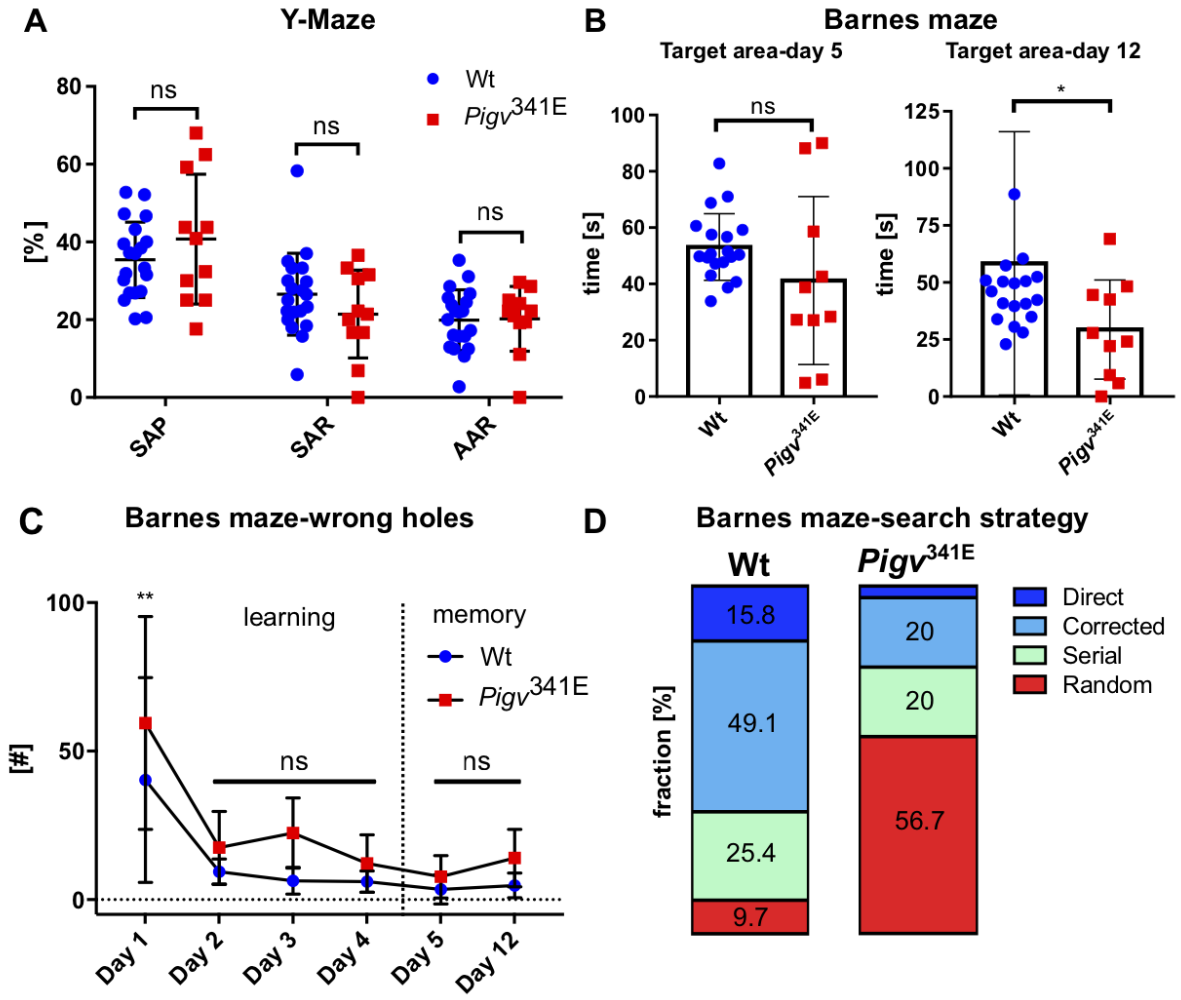
Supplementary figure 7



***Pigv*^{341E} mice exhibit a social phenotype and no defects in olfaction** (A) The ratio between empty cage and stranger 1 was increased (left graph) in *Pigv*^{341E} mice indicating an enhanced social approach behavior in the three-chamber test (first 5 minutes). Ratio between stranger 1 and stranger 2 (right graph) did not show any differences between genotypes in the three-chamber test (first 5 minutes). (B) During the habituation phase *Pigv*^{341E} and wild-type mice did not show any preferences for the left and right chamber (C) In the social proximity test, *Pigv*^{341E} mice revealed no significant differences to wild-type in numbers of "crawl over" (left graph) and "crawl under" (right graph) when interacting with the stranger mice. (D) In the buried food test, no differences were observed in olfaction abilities between genotypes.

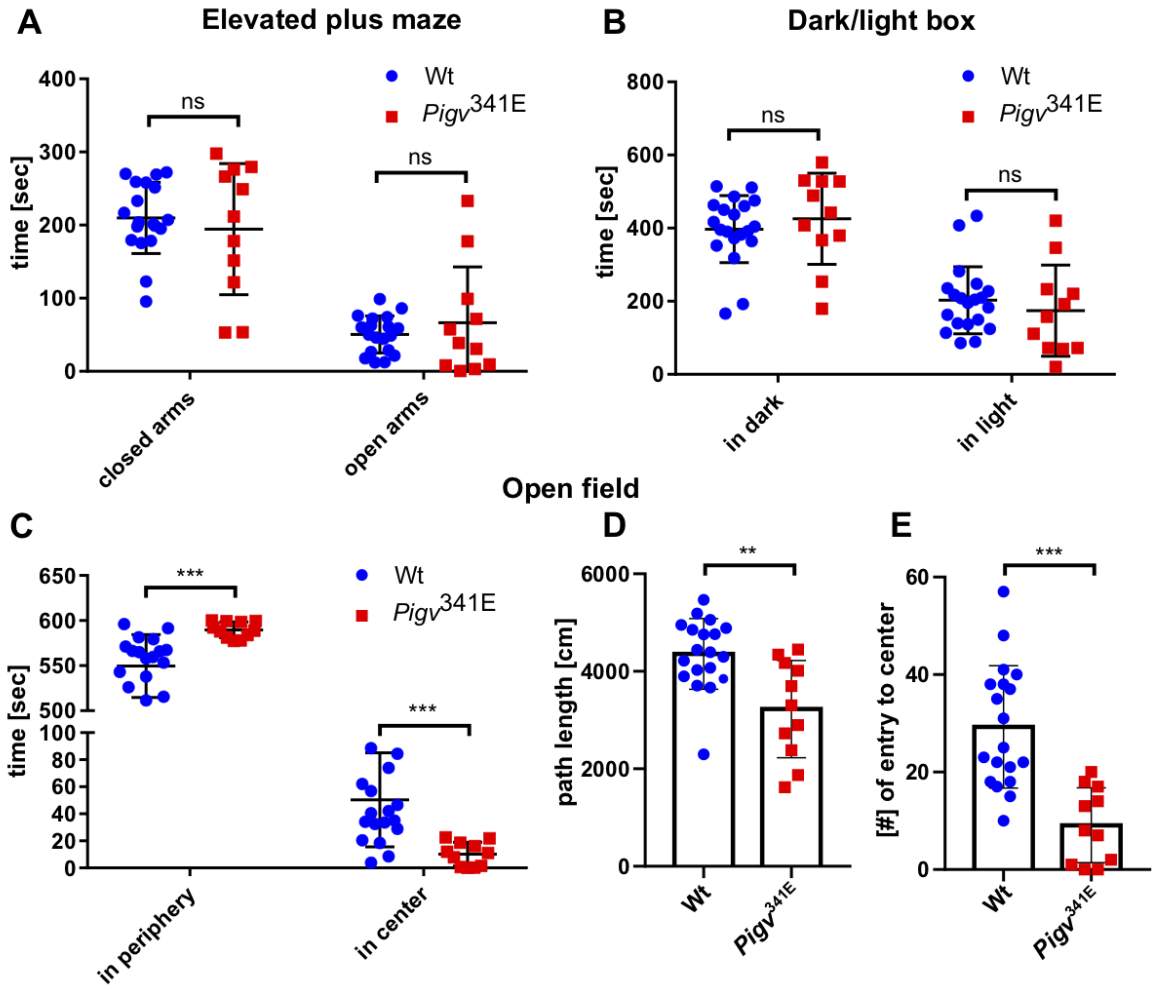
Pigv^{341E}=homozygous for Pigv p.Ala341Glu, wt=wild-type. Animals used for the three-chamber test: Wt(female n=9, male n=11), *Pigv*^{341E}(female n=4, male n=6). Stranger mice had the same age and sex. Animals used for the social proximity test and buried food test: Wt(female n=9, male n=11), *Pigv*^{341E}(female n=4, male n=7). The data from the social proximity test, three-chamber test (ratio strainger2/1, stranger1/empty) and buried food test was analyzed with a non-parametric t-test (Mann-Whitney). The data from the three-chamber test (habituation phase) was analyzed by two-way of variance (ANOVA) followed by a Bonferroni's multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001.

Supplementary figure 8



***Pigv*^{341E} mice showed cognitive deficits in spatial memory.** (A) In the y-maze, *Pigv*^{341E} mice showed no alterations in spontaneous alternation performance (SAP), same arm returns (SAR) and alternate arm returns (AAR) and thus are not impaired in short-term working memory. (B) *Pigv*^{341E} mice spent less time in the target quadrant of the Barnes maze at day 12 (long-term memory). At day 5 (short-term memory) there was no significant difference between genotypes. (C) In the Barnes maze, *Pigv*^{341E} mice showed no significant differences in the number of wrong holes to wild-type during day 2-12. (D) Fractions of search strategies during the Barnes maze test that *Pigv*^{341E} mice and wild-type used in order to find the correct exit hole were shifted in *Pigv*^{341E} mice. Direct=direct way to the correct exit hole, corrected=mice targeted first 1-3 wrong holes and went afterwards directly to the correct hole, serial=mice checked all holes in series, random=mice targeted random holes without any order.
Pigv^{341E}=homozygous for Pigv p.Ala341Glu, wt=wild-type. Animals used for the y-maze: Wt(female n=9, male n=11), *Pigv*^{341E}(female n=4, male n=7). Animals used for the Barnes maze: Wt(female n=8, male n=11) *Pigv*^{341E}(female n=4, male n=6). The data from the y-maze was analyzed with a non-parametric t-test (Mann-Whitney). The data from the Barnes maze (number of wrong holes) was analyzed by two-way of variance (ANOVA) followed by a Bonferroni's multiple comparisons test. *P<0.05 **P<0.01, ***P<0.001.

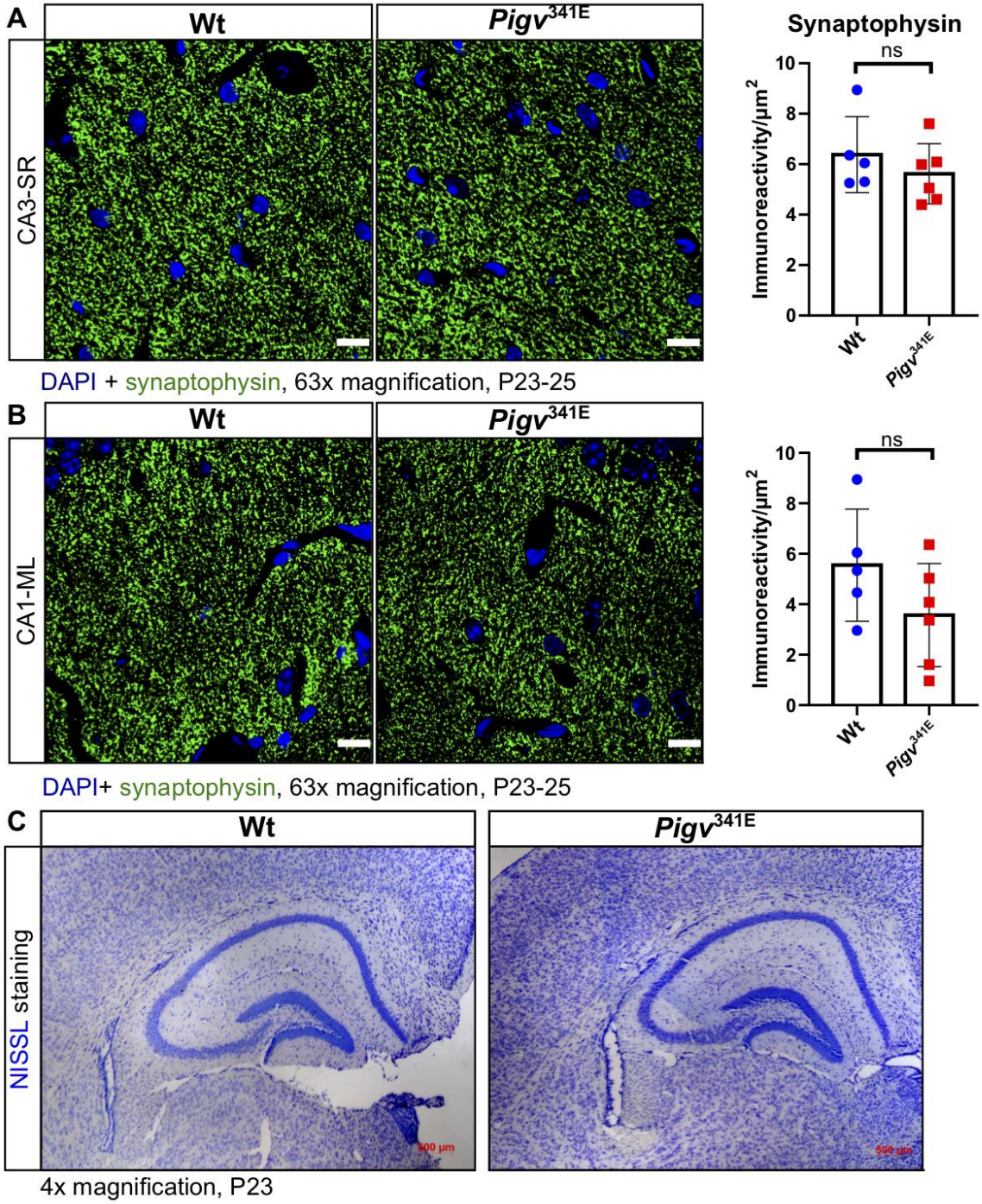
Supplementary figure 9



Affective behavior of *Pigv*^{341E} mice. (A) In the elevated plus maze, *Pigv*^{341E} mice showed no significant differences to wild-type in exploration behavior displayed by spending the same time in the open and closed arms. (B) In the dark/light box, *Pigv*^{341E} mice exhibited no significant differences to wild-type in exploring the dark and light compartment. (C) In the open field test, *Pigv*^{341E} mice spent more time in the periphery than in the center. (D) *Pigv*^{341E} mice showed a reduced distance traveled during the open field displayed by a reduced path length. (E) *Pigv*^{341E} mice visited less time the center part of the open field.

Pigv^{341E}=homozygous for Pigv p.Ala341Glu, wt=wild-type. Animals used for the the elevated plus maze, dark/light box: Wt(female n=9, male n=11) *Pigv*^{341E} (female n=4, male n=7). The data from the elevated plus maze, dark/light box, open field was analyzed by two-way of variance (ANOVA) followed by a Bonferroni's multiple comparisons test. The data from the open field (number of visits, path length) was analyzed with a non-parametric t-test (Mann-Whitney). *P<0.05 **P<0.01, ***P<0.001.

Supplementary figure 10



***Pigv*^{341E} mice showed no morphological abnormalities in the hippocampus** (A-B) Representative image of immunofluorenscence staining showed no differences of synaptophysin immunoreactivity (green) in *Pigv*^{341E} mice in CA3-SR and CA1-ML. DAPI (blue) (left). Images were taken at 63x magnification, scale=10 µm. Synaptophysin immunoreactivity were quantified with Fiji ImageJ and revealed no significantly reduced immunoreactivity/µm^2 in CA3-SR and CA1-ML between genotypes (right). Animals were 3 weeks old: Wt(female n=3, male n=2) *Pigv*^{341E}(female n=2, male n=4). (C) Representative images of a NISSL staining revealed no morphological abnormalities in the hippocampus between genotypes. 4x magnification, scale=500 µm.